AMENDMENTS TO SPECIFICATION

The paragraph that begins on page 3, line 4, has been amended as follows:

Higher density hybridization arrays have been made with a block of pins that were dipped into multiple wells at a time and then spotted on a membrane in an interspersed pattern ([[1]] Lehrach et al., 1990). However, this approach remains largely a manual procedure with variation in spot size and volume, and limitations on reproducible manufacture.

The paragraph that begins on page 3, line 9, has been amended as follows:

Higher density arrays of oligonucleotide probes were first developed by Fodor et al. ([[2]] 1991) and Pirrung et al. (U.S. Pat. No. 5,143,854), wherein nucleic acid segments were synthesized in place at the different array locations. These methods involved complex methods and equipment, and the probes generated were short (20 to 25 bases). A related method was described by Southern et al. ([[3]] 1992). Oligonucleotide arrays have also been described by Khrapko, et al. (1991) [[(4)]] in which DNA was hand spotted on a polyacrylamide gel with a micropipetter. However, oligonucleotide arrays were principally developed for detecting DNA sequences and only recently were they reapplied to make gene expression microarrays similar to cDNA-based microarrays (U.S. Pat. No. 6,040,138). In the past year, several biotechnology companies have begun making microarrays and sample components based on synthetic, long oligonucleotides suitable for printing.

The paragraph that begins on page 4, line 3, has been amended as follows:

The recent development of cDNA based expression microarrays provides a ready means to simultaneously assess the relative expression of hundreds or thousands of different genes from cellular or tissue samples ([[5-11]] Schena et

al., 1995, 1996; Shalon et al., 1996; DeRisi et al., 1996; Heller et al., 1997; Khan et al., 1998, 1999). These analyses were accomplished by first preparing miniature grids or arrays on membranes or coated glass substrates by spotting robotically small but dense cDNA samples of individual genes in a two dimensional pattern. Then, the mRNA transcripts of a sample were copied using reverse transcriptase, a poly-T primer and labeling agents to create a pool of cDNA based probes. These labeled probes were then hybridized to their respective gene spots in the expression microarray in order to detect and determine the relative frequency of each transcript in the original sample. These expression microarrays, which are also commonly called cDNA chips, DNA chips or BioChips, can also be manufactured from gene specific synthetic oligonucleotides that likewise were created or distributed on an array in a two dimensional pattern (U.S. Patent No. 5,445,934, U.S. Patent No. 5,800,992 and U.S. Patent No. 6,040,138).

The paragraph that begins on page 4, line 20, has been amended as follows:

In high density gene expression microarrays, picoliter quantities of cDNA-based reagents are deposited in close proximity on solid supports, with assay spots typically 75 to 150 microns in diameter and with center to center (CTC) spacing of 100 to 375 microns. Most of these devices spot the microarrays with a row of fine pins that load and dispense by capillary action. The pins may be solid, split, pinched like a quill pen, scored with channels, or encircled with a floating ring to hold larger quantities of sample per loading and to allow dispensing a defined smaller sample per spot. Typically, 0.5–2.5 nanoliter quantities are dispensed by capillary action as the spring-loaded pin is brought into contact with the substrate surface (US Patent No. 6,110,426). Alternatively, piezoelectric or inkjet technology has been employed to load larger quantities and to dispense small microdroplets by electronic activation without contacting the surface. Gamble et al. reported a piezoelectric or thermally activated pulse

jetting device where the tip of the print head or pulse jet can dispense microdroplets of about [[0.05]] 0.5 nanoliters or less to achieve spots on a chip spaced 80 microns CTC with 15 microns between spots (US Patent No. 6,001,309).

The paragraph that begins on page 5, line 17, has been amended as follows:

The technologies described above were intentionally developed to create very high-density microarrays with thousands of different gene specific assay spots per chip. The need to miniaturize these expression arrays is due to the fact that mRNA samples available for such analyses are frequently quite limited and methods to amplify the sample products are inefficient. Moreover, hybridization kinetics is very slow in large volumes of hybridization solution. Therefore, in order to assess the expression of hundreds or thousands of genes per sample, the spot density of the array format must be greatly increased without increasing the size dimensions of the array, the volume of hybridization solution, the time of hybridization, or the overall sensitivity for detecting each analyte in the sample. However, the development of these miniaturized, high density arrays comes at great cost and limitations, since the equipment required is complex and delicate, the pin heads or jets must be thoroughly washed and cleaned between sample loadings, specialized temperature and humidity controls and enclosures are required, and complex robotic procedures must be programmed for each run. Very high-density microarrays must also employ dust free "clean room" conditions and equipment that parallel the specialized facilities required for the manufacture of computer chips. Such miniaturization also requires the use of very expensive, specialized labeling reagents. Moreover, while these expression microarrays allow a high throughput overview and assessment of the relative frequency of different gene transcripts in a sample, these methods are limited by significant deficiencies in

quantification and sensitivity (~~8, 12-13~~ DeRisi et al., 1996; Duggan et al., 1999; Rajeevan et al., 1999).

The paragraph that begins on page 7, line 1, has been amended as follows:

One approach to improve chip detection would be to amplify mRNA derived probes by the polymerase chain reaction (PCR) or related enzymatic methods. However, effective multi-analyte amplification typically requires the provision of at least one unique primer for each type of gene product amplified, and commonly available PCR procedures such as RT-PCR and multiplex PCR have only been used successfully to amplify a limited number of the gene products in a sample (U.S. Pat. No. 5,807,680). In methods such as differential display or other older procedures that are used to explore expression differences, global amplification methods have been employed based upon using simple arbitrary primers, hexamers or various random primer constructs instead of unique primers to amplify DNA or RNA. Inconsistency of these methods renders them useful only for identifying unusual or novel gene expression products, and they have not been devised or employed for use with expression microarrays or DNA chip analyses (~~14-21~~ Liang et al., 1993; Mou et al., 1994; Welsh et al., 1990; U.S. Patent Nos. 5,262,311; 5,665,547; 5,580,726; 5,104,792; 5,789,206; 5,882,856).

The paragraph that begins on page 7, line 16, has been amended as follows:

The prime difficulty with many of these amplification methods stems from the use of short arbitrary or random primers that can give variable results from gene to gene under different temperature and hybridization conditions such that they are unsuitable for repeated diagnostic analyses. Even RT-PCR or multiplex PCR methods which employ unique primers can produce semi-quantitative rather than quantitative results because different primer sets vary

considerably in efficiency. Moreover, kinetic factors favor copying the smaller and more abundant products in these methods. Therefore, some products may not amplify well, and rare or down-regulated transcripts may be under-represented (~~11~~ Khan et al., 1999). Additionally, mammalian mRNA samples include very large gene transcripts 6 to 12 thousand nucleotides long that cannot be amplified reliably by routine PCR methods. Consequently, global PCR amplification of a pool of mRNA-derived cDNA probes has not been attempted or successfully accomplished with DNA chip or expression microarray analyses. Based on the above reasons, currently available exponential amplification methods cannot be validly applied to multi-analyte gene expression analysis.

The paragraph that begins on page 8, line 13, has been amended as follows:

Less robust linear amplification methods have been developed and employed for chip analyses by adding a RNA polymerase promoter to the end of the poly-T primer used for RT. However, such amplification is incremental and finite, with a typical duplication of 20-60 copies, and the amplified products it produces are antisense RNAs which are degradable (~~22-24~~ Philips et al., 1996; U.S. Patent Nos. 5,972,607; 5,716,785). Wang et al. (U.S. Pat. No. 5,932,451) refined such methods to allow asymmetrical amplification of double stranded cDNA made from a mRNA sample. However, this amplification method is also limited in the number of copies typically made from a sample (only 68 fold duplication demonstrated).

The references listed on page 51, line 11 to page 52, line 16 have been amended as follows:

~~1. Lehrach et al., "Hybridization Fingerprinting in Genome Mapping and Sequencing," in Genome Analysis, vol. I: Genetic and Physical Mapping.~~

(K.E. Davies & S.M. Tilgham, Eds.) Cold Spring Harbor Laboratory Press, pp. 39-81 (1990).

2. Fodor et al., Science, 251:767-773 (1991).

3. Southern et al., Genomics, 13:1008-1017 (1992).

4. Khrapko et al., DNA Sequencing and Mapping, 1:375-388 (1991).

5. Schena et al., Science, 270: 467-470 (1995).

6. Schena, et al., Proc. Natl. Acad. Sci., 93:10614-9 (1996).

7. Shalon et al., Genome Res., 6: 639-45 (1996).

8. DeRisi et al., Nature Genetics, 14: 457-60, (1996).

9. Heller et al., Proc. Natl. Acad. Sci., 94: 2150-5, (1997).

10. Khan et al., Cancer Res., 58: 5009-13 (1998).

11. Khan et al., Electrophoresis, 20: 223-9 (1999).

12. Duggan et al., Nature Genetics, 21: 10-14 (1999).

13. Rajeevan et al., Jour. Histochem. Cytochem., 47: 337-42 (1999).

14. Welsh et al., Nucleic Acids Res., 18: 7213-18 (1990).

15. Pardee et. Al., U.S. Pat. No. 5,262,311 (1993) and 5,665,547 (1997).

16. Liang et al., Nucleic Acids Res., 21: 3269 (1993).

17. Mou et al., Biochem. Biophys. Res. Comm., 199: 564-569 (1994).

18. Villeponteau et al., U.S. Pat. No. 5,580,726, (1996).

19. Silver et. Al., U.S. Pat. No. 5,104,792 (1992).

20. Tavtigian et al., U.S. Pat. No. 5,789,206 (1998).

21. Shuber, U.S. Pat. No. 5,882,856 (1999).

22. Phillips et. Al., Methods, 10: 283-288 (1996).

23. Kondo et al., U.S. Pat. No. 5,972,607.

24. VanGelder et al., U.S. Pat. No. 5,716,785 (1998).

DeRisi et al., Nature Genetics, 14: 457-60, (1996).

Duggan et al., Nature Genetics, 21: 10-14 (1999).

Fodor et al., Science, 251:767-773 (1991).

Heller et al., Proc. Natl. Acad. Sci., 94: 2150-5, (1997).

Khan et al., Cancer Res., 58: 5009-13 (1998).

Khan et al., Electrophoresis, 20: 223-9 (1999).

Khrapko et al., DNA Sequencing and Mapping, 1:375-388 (1991).

Lehrach et al., "Hybridization Fingerprinting in Genome Mapping and Sequencing," in Genome Analysis, vol. I: Genetic and Physical Mapping. (K.E. Davies & S.M. Tilgham, Eds.) Cold Spring Harbor Laboratory Press, pp. 39-81 (1990).

Liang et al., Nucleic Acids Res., 21: 3269 (1993).

Mou et al., Biochem. Biophys. Res. Comm., 199: 564-569 (1994).

Phillips et al., Methods, 10: 283-288 (1996).

Rajeevan et al., Jour. Histochem. Cytochem., 47: 337-42 (1999).

Schena et al., Science, 270: 467-470 (1995).

Schena, et al., Proc. Natl. Acad. Sci., 93:10614-9 (1996).

Shalon et al., Genome Res., 6: 639-45 (1996).

Southern et al., Genomics, 13:1008-1017 (1992).

Welsh et al., Nucleic Acids Res., 18: 7213-18 (1990).